(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 June 2001 (14.06.2001)

PCT

(10) International Publication Number
WO 01/41827 A1

(51) International Patent Classification[7]: A61L 27/34, 27/28

(21) International Application Number: PCT/GB00/04685

(22) International Filing Date: 7 December 2000 (07.12.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
9928956.3 7 December 1999 (07.12.1999) GB

(71) Applicants and
(72) Inventors: **MALIK, Navid** [GB/GB]; 1 Lynton Road, Kilburn, London NW6 6BD (GB). **EVAGOROU, Evagoras** [GB/GB]; 112 Park Lane, Tottenham, London N17 0JP (GB).

(74) Agent: **WHITE, Duncan, Rohan**; Reginald W. Barker & Co., Clifford's Inn, Fetter Lane, London EC4A 1BZ (GB).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/41827 A1

(54) Title: INTERNALLY SUPPORTED BIOMIMETIC MATERIALS AND COATINGS

(57) Abstract: A material comprising a combination of a biomimetic material and a substance adapted to allow the biomimetic material to adopt the appropriate structural orientation to achieve maximum efficacy as a biomimetic. The substance comprising a branched polymer which supports the biomimetic material internally. Examples of biomimetic materials include phosphorylcholine, a polysaccharide, a mucopolysaccharide or a glycosaminoglycan, syalic acid, or lectin, or a derivative or analogue of any of said biomimetic materials.

INTERNALLY SUPPORTED BIOMIMETIC MATERIALS AND COATINGS

In the past, a number of devices employed for medical use were based on their durability with less concern about their impact on the tissues immediately surrounding the device. Since these devices would be in place for periods of days to years, the subsequent biological concerns were justified. As experience grew, and medical complications and costs became an issue (particularly those issues relating to regulatory approval), a number of investigators directed their attention to the biological responses surrounding inflammation and immunogenicity to the device or a component of it and its soilation.

Today a number of state of the art medical devices or materials are available including but not restricted to polypropylene, polyvinyl chloride (PVC), polytetrafluoroethylene (PTFE), polyesters, polyurethanes, polyamides, vinylic, polycarbonate, acrylic polymers and stainless steel. As improvements are continuously being made, these devices will last long-beyond the lifetime of their predecessors. Additionally, in order to prolong the lifetime it is becoming more common for these materials to be specially prepared or treated with substances that are inert or that will induce a minimal biological response, leading to safer and more tolerable technologies.

There is still however a real and immediate need to provide medical devices and implants that *do not* elicit a significant biological response once in position. A biological attack stems from the adsorption of proteins or platelets from bodily fluids (e.g. plasma) onto the medical device or implant; examples being intraocular lenses (contact lenses), arterial stenting supports (in the treatment of atherosclerosis), artificial limbs and organs, and prosthetic attachments. Furthermore, interaction of biomaterials with a range of blood components including neutrophils is responsible for some of the clinical complications that have occurred in therapeutic treatments such as cardiopulmonary bypass, hemodialysis, and ventricular assist procedures. The possibility of inhibiting the initial adhesion of neutrophils to biomaterials has been studied extensively, but many of the problems, some serious, remain unsolved.

Biological attack of a material from the host (e.g. protein or fibrinogen binding) is known to be based on significant interfacial problems i.e. the surface of the material is not biologically inert and has the ability to induce a host response. Consequently, these problems relate to material efficiency and functional lifetime (due to soilation for example). Other

reactions can exist at a local level e.g. lysozyme activity towards an ocular device that is present in the eye portal. These problems are further compounded by the fact that deposition of protein stimulates attachment of fibrous and antibiotic moieties, leading to subsequent biological responses.

The extent of protein absorption is dependent on a number of factors relating to the outer surface of the device, including but not restricted to hydrophobicity, electrostatic interactions and to some extent size. It is known that hydrophilic surfaces tend to reduce protein binding through adsorption. Thus some linear polymers such as polyethylene oxide (PEO) and PTFE do not elicit a large response but evidently that which is observed is believed to be unacceptable. A cumulative response over time with hydrophilic systems is eventually enough to lead to adverse effects as presented by more hydrophobic devices. Thus, coating such devices with a 'material' which reduces or eradicates any biological response is advantageous. Even more so, it is desirable to use a 'material' which is found within the biological domain so that more serious issues in the long-term can be negated. Ideally, the 'material' can be transferred from technology to technology.

A number of such materials have been under investigation. What has become a common feature to virtually all such materials is that they are derived from a natural origin. Some of these materials will be described individually.

One such 'material' is a derivative of the naturally occurring group of phospholipids, called phosphorylcholine (PC). In cholinergic neurons choline is directed to a number of pathways, these include (1) conversion to phosphorylcholine and cytidine diphosphate choline (CDP-choline) for the synthesis of phosphatidylcholine and (2) acylation to the neurotransmitter acetylcholine.

In vivo lysosomal breakdown of glycosphingolipids with short hydrophilic carbohydrate headgroups is achieved by the simultaneous action of specific hydrolases and sphingolipid activator proteins (SAPs). Activator proteins are considered to facilitate the enzyme/substrate interaction between water-soluble enzymes and membrane-bound substrates. Sphingomyelin, containing the small hydrophilic phosphorylcholine moiety, is hydrolysed by acid sphingomyelinase (acid SMase).

Sphingomyelin (N-acylsphingosin-1-phosphorylcholine) is a phospholipid preferentially found in the plasma membrane of mammalian cells. Signalling through the

sphingomyelin pathway is associated with generation of ceramide, which acts as a second messenger in activating a variety of cellular functions. Ceramide belongs to the group of sphingosine-based lipid second messenger molecules that are critically involved in the regulation of signal transduction of diverse cell surface membrane receptors. The emerging picture suggests that coupling of ceramide to specific signalling cascades is both stimulus- and cell type-specific and depends on the subcellular topology of its production. Following membrane receptor triggering, neutral and acid isoforms of sphingomyelinases are rapidly activated generating ceramide through sphingomyelin hydrolysis.

The 'BiodivYsio stent' is a stent coated with PC, a biocompatible molecule, used in this case, to prevent a biological response to the metallic stent after catheter based interventions in chronic obstructive arterial disease, and hence the potential risk of restenosis initiated by iatrogenous endothilial injury promoted by growth factors. The feasibility, safety, and efficacy of elective and urgent implantation of this coated coronary stent were prospectively studied in a paper by Zheng *et al.* (1999) using a number of human patients. This initial clinical experience indicates that the implantation of stents coated with phosphorylcholine appears to be safe and efficacious in the treatment of complex coronary lesions and is associated with an extremely low target vessel revascularization rate.

A study on the arterial wall reaction to phosphorylcholine-coated metal stents was examined in rabbits and pigs. Compared to non-coated stents, no significant difference was found by angiography and histology. The authors concluded that although phosphorylcholine-coating did not provoke arterial neointima formation or decrease luminal diameter compared to stainless steel stents, the coating did not seem to reduce the risk of restenosis (Kuiper *et al.*, 1998).

Lim *et al.*, (1999) examined *in vitro* whether phosphorylcholine coating of poly(methylmethacrylate) (abbreviated as PMMA) could reduce the adhesion of fibrinogen, fibrin, human scleral fibroblast and macrophage compared with current biomaterials that were used in the construction of glaucoma drainage devices. Sample discs (n=6) of poly(methylmethacrylate), silicone, polypropylene, PTFE, and phosphorylcholine coated poly(methylmethacrylate) were seeded with fibrinogen, fibrin, fibroblast, and macrophages and incubated for variable lengths of time. The quantification was performed using radioactivity, spectrophotometry, ATP dependent luminometry, and immunohistochemistry

respectively. Results indicated that fibrinogen and fibrin adhesion to phosphorylcholine coated poly(methylmethacrylate) were significantly lower than PMMA ($p=0.004$). Phosphorylcholine coating of poly (methylmethacrylate) also significantly reduced the adhesion of human scleral fibroblast ($p=0.002$) and macrophage ($p=0.01$) compared with PMMA. All the other biomaterials showed either similar or insignificantly different levels of adhesion to all the proteins and cells tested compared with PMMA. Phosphorylcholine coating was therefore described as a new material technology that offered considerable promise in the field of glaucoma drainage device development.

To date, coatings such as phosphorylcholine has been combined with linear polymers, nanoparticles and microparticles. Linear polymers suffer from a number of disadvantages in relation to their ability to interact with and carry other therapeutic molecules or ligands. All of these systems suffer from similar drawbacks in relation to their synthesis and consequent combination with other ligands. There is a certain degree of polydispersity inherent in these systems, which leads to a number of problems, not only in their physico-chemical properties but also if they were combined with a pharmaceutically active agent, a problem relating to pharmacokinetics and pharmacodynamics should such a pharmaceutical agent need to be released at a later stage. In addition, the random coil nature of linear polymers relates a difficulty in knowing the percentage of terminal groups that are exposed at the outer surface rather than facing inwards into the coil, or indeed embedded in the coil.

Previously the disclosure of ArtiCell™ (WO-A-98/56353) described the use of branched polymers as a stable support for a lipid-based coating.

It is an object of the present invention to provide a new biomimetic material or coating. It is a further object of the present invention to reduce the above mentioned problems exhibited by prior art materials or coatings.

A material comprising a combination of a biomimetic material and a substance adapted to allow the biomimetic material to adopt the appropriate structural orientation to achieve maximum efficacy as a biomimetic.

The invention provides a material comprising a branched polymer supporting a biomimetic material. The biomimetic material is preferably at least one of phosphorylcholine, a polysaccharide, a mucopolysaccharide or a glycosaminoglycan or a derivative thereof. The branched polymer is preferably a dendrimer. Unlike linear polymers,

dendrimers have surface groups that are structurally forced to be out-facing. Combined with their very low polydispersity (being almost monodisperse compared to that of linear systems), the structural orientation of the dendrons ensures the provision of a 'stable' support. Therefore, such architectures provide an ideal support for a coating such as phosphorylcholine. The uniformity or smoothness of the coating is also known to be extremely important as this will lead to a reduction in imperfections at the surface, which might facilitate protein adsorption. A closely packed polymer film of for example phosphorylcholine can facilitate this. Therefore the symmetry of the surface groups present on branched polymers, which might include but is not restricted to a dendrimer, allows a uniform coating. In the branched polymer-biomimetic material of the invention the branched poymer such as a dendrimer, acts as a "structural support" or "scaffold" whereby the biomimetic material such as PC is allowed (forced) to adopt the appropriate and necessary structural orientation to achieve maximal efficacy.

The invention further provides a system comprising a branched polymer acting as a support for phosphorylcholine, polysaccharide, mucopolysaccharide, or glycosaminoglycan

The polysaccharide may be cellulose, starch, maltose, dextrin. Dextran, an algin or alginate, or a derivatives thereof.

The mucopolysaccharide may be chitin, chitosan or a derivative thereof.

The glycosaminoglycan may be hyaluronic acid, chondroitin, dermatan, keratin, or heparin, or derivatives thereof. The glycosaminoglycan may be chondroitin sulphate A, dermatan sulphate, keratin sulphate, or heparin sulphate.

The derivative or analogue of phosphorylcholine may be ceramide. The phosphorylcholine is an antigen or epitope.

The branched polymer may be a dendrimer, an arborol, a cascade polymer, a tubular polymer, a star polymer, a hyperbranched polymer, or a hyper comb-branched polymer.

The branched polymer may be cross-linked and used as the support for phosphorylcholine.

The invention also provides a hydrogel formed from a combination of a branched polymer and a biomimetic material. The branched polymer acts as the support for the coating of the biomimetic material which may be phosphorylcholine.

The ratio of surface or interior groups on the branched polymer to molecules of the biomimetic material (e.g. phosphorylcholine, an analogue or derivative thereof) may be 1:1 or any other ratio.

The biomimetic material forms a complete enclosure or an incomplete enclosure of the branched polymer.

The molecular weight of the branched polymer may be between 500 Da to 1 million Da but is not restricted thereto.

The phosphorylcholine may be synthetic or natural and may be derived from prokaryotes or eukaryotes.

A spacer/linker may be introduced between the branched polymer and the coating. The spacer/linker may be a peptide, or a polymer such as polyethylene glycol. The peptide may be mono-, di/bi-, tri-, tetra- peptide, but is not restricted thereto.

The material of the invention may be used to coat or interact with a medical device.

A functional group of the branched polymer may be exposed so as to facilitate attachment to another surface which include but are not restricted to a metal or polymer surface. A chain may be attached to an exposed functional group on the branched polymer or coating and is used to facilitate the attachment to another surface (including a medical device or implant) which include but are not restricted to a metal or polymer surface.

The medical device may be an ocular or intraocular lens, a stent, an artificial organ, or prosthetic device (including, but not restricted to, pacemaker leads, artificial heart valves, vascular grafts) or limb. The medical device may also be a glaucoma drainage device, a dialysis membrane or ultracentrifugation membrane, a thoracic drain catheter, a vascular graft, a urological catheter or device, a guidewire, an introducer sheath, a extracorporeal circuit component (including but not restricted to arterial filters and heat exchangers), or a hypodermic syringe needle.

The material or supported coating may be used for a non-medical use which might include an industrial or agroindustrial (agrochemical) process.

The material may be used to coat or (interact) covalently bind with the surface of an implant (biodegradable or non biodegradable).

The association between the branched polymer and the coating is covalent, van der waals, hydrophobic, electrostatic, or co-ordinate, neutral, or hydrogen bonding

The invention further provides a combination including a material according to the invention and a pharmaceutically active agent reversibly attached to the coating layer. Alternatively, a pharmaceutically active agent may be reversibly attached to the branched polymer.

The reaction forming the coating on the support may be conducted in an aqueous solution or a solvent or a mixture.

The supporting material and/or biomimetic material may be a solution, a gel, a solid, a film, a casting, a fibre or fabric.

The material may be provided in the form of or in combination with a solution, a gel, a solid, a film, a casting, a fibre or fabric. The material/system may be in the form of an adhesion prevention composition. The material/system may be soluble, insoluble, or biodegradable in an aqueous solution or another solvent (organic or otherwise).

The material of the invention may be used to support other materials such as sialic acid, or lectin, or derivatives thereof.

In the present invention, a new coating, the ArtiCoat™, is revealed which has a multitude of biomedical and industrial uses.

Phospholipids are an important constituent of the cell membrane. Removal of the lipid (hydrophobic) component of the phospholipid leaves the phosphate based hydrophilic component that is known as phosphorylcholine. For those experienced in the art, incorporation of phosphorylcholine on the outer surface of the material or device will act as a biomimetic membrane that will be biocompatible and not likely to cause an immunogenic response.

The invention provides in one aspect the system comprising a branched polymeric structure, which provides a structural support for a phosphorylcholine, polysaccharide, mucosaccharide or glycosaminolycan coating. The support and coating acting as a biomimetic.

The invention further provides a drug delivery system.

The invention will now be described in more detail with reference to examples. In particular examples of the compositions which may be used as the branched polymer or the biomimetic material will be discussed.

Figure 1 shows the chemical structure of materials which may be used as biomimetic materials.

Polysaccharides

Carbohydrates are characterised by the presence of polyhydroxylic aldehyde or polyhydroxylic ketone structures or polymers of such units.

Monosaccharide, the simplest carbohydrate, contains a number of functional groups, which may be modified or used for conjugation. These include a ketone or an aldehyde, hydroxyl groups, and the possibility of amine, carboxylate, sulphate, or phosphate groups as additional substituents. Amine-containing sugars may possess a free primary amine, but naturally are often found as the *N*-acetyl derivative, such as *N*-acetylglucosamine residue of chitin.

Polysaccharides are formed by the condensation of large numbers of monosaccharide units which are joined together with the elimination of water much in the same way as the amino acids are joined together to form proteins. Like proteins, polysaccharides have high molecular weights and are commonly insoluble in water or form colloidal solutions. The three chief polysaccharides, starch, glycogen and cellulose, are built up solely from glucose units.

Starch is the form in which carbohydrate is stored in plants. It occurs in plant tissues, for example potato, in the form of granules with a thin cellulose coating. These granules themselves are insoluble in water although at boiling temperatures the coating ruptures and the starch is liberated forming a colloidal solution with an opalescent appearance. Partial hydrolysis yields maltose. Starch contains one-quarter amylose and three-quarters amylopectin. Amylose consists of long unbranched chains. It gives an intense blue colour with iodine. Amylopectin, on the other hand, contains more than 1000 glucose residues in a highly branched structure with about 24 to 30 units per branch. While most units are joined by α-linkages between carbon 1 of the unit and carbon 6 of the next, the branches start as α-linkages between carbon 1 of one unit and carbon 6 of the unit in the chain from which the branch originates. Amylopectin gives a brown colour with iodine.

Cellulose is a very stable polysaccharide which forms the supporting tissues of the plant. It is insoluble in water and gives no colour with iodine. Although ingested in

considerable quantities, it is not digested to any extent by man. Herbivorous animals, however, are able to make use of cellulose since the bacteria and protozoa in the rumen or colon convert it, not to glucose, but to small fragments such as short-chain fatty acids, carbon dioxide and methane. The cellulose molecule consists of very long chains of glucose units in the β-configuration joined together by 1,4-linkages.

Mucopolysaccharide

The mucopolysaccharides are a group of complex and biologically important materials which exist in nature combined with small, but significant, amounts of protein. The units composing them include, as well as hexoses and pentoses, either uronic acids or hexosamines, or both. The polyuronides are composed of uronic acid units and are found in amongst other species plants and bacteria.

The polyhexosamines include chitin which forms the shells of crustaceans and the hard outer portions of insects. It is composed of glucosamine units joined together by β-linkages and it is therefore very similar in structure to cellulose. The hexosamines, together with simple hexoses, are constituents of the polysaccharides of the specific blood group substances found in the red blood cells.

Although mentioned in the next section owing to its chemical structure, hyaluronic acid falls into the category of mucopolysaccharides also, as it is found in the vitreous humor of the eye, the umbilical cord and synovial fluid.

Glycosaminoglycans

Glycosaminoglycans are a class of natural, water-soluble polysaccharide polymers found in animal connective tissues and in the blood. They are highly charged polyanions and are most favourable as drug carriers as they are highly biocompatible and non-immunogenic. Well known examples are the chondroitins and their sulfate derivatives, heparin, hyaluronic acid, keratan and its sulfate derivative and dermatan and its sulfate derivative.

Endothelial cells have two major roles in homeostasis. The first is to provide an inert surface that does not activate coagulation of circulating blood. The second is to promote homeostatic processes when vascular surfaces are damaged. The haemostatically 'inert' surface appears to depend on a negative electrical charge on the surface of the cells. This is

provided by the glycosaminoglycans of the cell coat or *glycocalyx*. Heparin is found in mast cells. It binds to antithrombin III (a plasma antiprotease which is important in homeostasis as it is the most important natural inhibitor of coagulation) and greatly increases its inhibitory activity. Heparin is used clinically-as an anticoagulant.

Other than heparin, one of the most important glycosaminoglycans is hyaluronic acid (more appropriately known as hyaluronan) which consists of alternating residues of N-acetylglucosamine and glucuronic acid. It forms aqueous solutions of high viscosity and is found for example in the skin, in the vitreous humour of the eye, in the umbilical cord and in certain bacteria. Amongst its roles it provides a cementing function in the tissues and the capillary wall, and it forms a coating gel around the ovum. More appropriate to this invention is the ability of hyaluronan in inhibiting cell-cell adhesion.

Chondroitin sulphate A is an acid mucopolysaccharide, which is a constituent of most mammalian cartilaginous tissues. It is often given to patients with ischaemic heart disease.

Algins and alginates

Algin or alginic acid (or D-mannuronic acid), is a mucilaginous carbohydrate substance derived from certain algae or seaweed, primarily brown seaweed. Used as the sodium salt, a very viscous solution is formed with water and a major function is as stabilisers or as food thickening products as well as compositions in pharmaceuticals. Alginate (containing mannuronic (M) and/ or guluronic (G) acid) is also a carbohydrate obtained from brown marine rockweeds (algae) and used for its emulsifying properties. Algins and alginates have been used as artificial threads, plastic material, films, gels, vulcanites. Biomedical uses are owed to their biocompatibility and include, among other uses, drug delivery, implantable materials, wound treatment and bandage dressings.

1. Synthesis of the internal support
Dendrimer (cascade polymer, hyperbranched polymer, arborol)

The methods described for the synthesis of dendrimers have been previously described in the literature.

Dendrimers possess three structural features, which afford them their unique and distinctive properties (structural or otherwise). They have an initiator core, interior areas,

which have cascading tiers or branch cells with radial connectivity to the initiator core and an exterior or surface region of terminal moieties attached to the outermost generation.

Two general methods have been proposed to synthesise a dendrimer. The divergent route where synthesis begins from the core, or the convergent route where synthesis begins from the terminal groups. In addition, one step synthesis can be employed or multi-step in the formation of the dendritic structure.

Divergent dendritic construction results from sequential monomer addition beginning from a core and proceeding outward toward the macromolecular surface. To a respective core representing the zeroth generation and possessing one or more reactive site(s), a generation or layer of monomeric building blocks is covalently connected. Repetitive addition of similar, or for that matter dissimilar, building blocks (usually effected by a protection-deprotection scheme) affords successive generations. A key feature of the divergent method is the exponentially increasing number of reactions that are required for the attachment of each subsequent tier (layer or generation).

The convergent dendritic construction is a strategy whereby branched polymeric arms (dendrons) are synthesised from the "outside-in". This concept can be best described by envisioning the attachment of two terminal units containing a reactive group to one monomer possessing a protected functionality, resulting in the preparation of the first generation or tier. Transformation of the active or focal site followed by treatment with 0.5 equivalent of the masked monomer affords the next higher generation.

One-step hyperbranched polymers are synthesised by a direct one-step polycondensation of A_xB monomers, where $x \geq 2$. Graft-on-graft procedure (chloromethylation followed by anionic grafting) has been used to synthesise tree-like structures.

At least 150 families of dendrimers have been synthesised and recorded in the literature over the past decade or so. In this respect it is impossible to describe every possible method of synthesis. Many more dendrimers are becoming commercially available.

Owing to the exhaustive synthetic possibilities only the two main routes will be described here, both methods have been described in the literature.

Synthetic Methodologies:

i) Divergent procedures

Example 1: Synthesis of polyamidoamine dendrimers (Tomalia *et. al*, 1985)

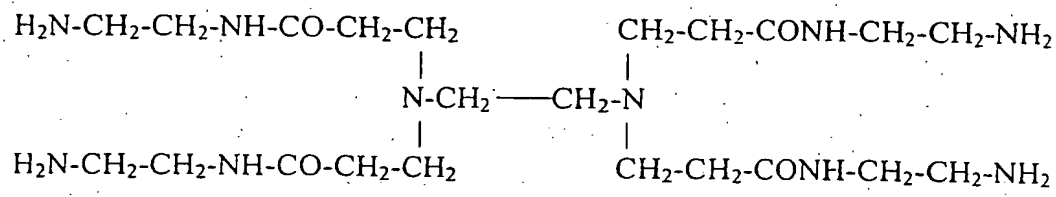
Polyamidoamine (PAMAM™) dendrimers are obtained by an alternating sequential Michael's addition reaction of ethylenediamine (H_2N-CH_2-CH_2-NH_2) and methyl acrylate (H_2C=CH-$COOCH_3$) to produce a methyl ester (half generation, carboxy terminated). Further addition with ethylenediamine produces the full generation (amine terminated) and extension of the dendritic branching. A purification step is incorporated into the reaction to achieve selectivity for size. The reaction is shown below:

1. H_2N-CH_2-CH_2-NH_2 + 4 H_2C=CH-$COOCH_3$ →

$(H_3COOC\text{-}H_2C\text{-}CH_2)_2NCH_2\text{-}CH_2N(H_2C\text{-}CH_2\text{-}COOCH_3)_2$

GENERATION -0.5

2. $(H_3COOC\text{-}CH_2\text{-}CH_2)_2$ $NCH_2\text{-}CH_2N(H_2C\text{-}CH_2\text{-}COOCH_3)_2$ + 4 $H_2N\text{-}CH_2\text{-}CH_2NH_2$ →



GENERATION 0

Generally as the reaction proceeds the number of functional groups at the terminus is doubled. Successive generations or half generations are synthesised by repeating the steps with an excess of the monomer, and incorporating a purification and characterisation step at each stage of synthesis.

Example 2: Synthesis of nitrile and carboxylate terminated dendrimers (Meijer *et. al*, 1993)

The synthesis of poly (propylene imine) dendrimers from a diaminobutane core was carried out using Michael's addition of acrylonitrile to primary amines, followed by heterogeneously catalysed hydrogenation of the nitriles, resulting in the doubling of the number of primary amines. 1,4-diaminobutane was used as a core; a number of molecules with either primary or secondary amine groups can also be used. All Michael's reactions were performed using 2.5-4.5 equivalents of acrylonitrile per primary amine at a concentration of 0.1 M in aqueous solution. The first equivalent of acrylonitrile was added at room temperature and the second equivalent at 80°C. The reaction time for the complete conversion increased with every generation: 1 h for generation 0.5 (DAB-dendr-$(CN)_4$), 3 h for generation 4.5 (DAB-dendr-$(CN)_{64}$). The excess of acrylonitrile was distilled off as a water azetrope. The two-phase clear system obtained allowed the isolation of pure dendrimers with nitrile terminations after pouring off the aqueous layer. The impurities (monomer) were removed by washing the residue with distilled water. Hydrogenation of the cyanoethylated product with H_2 (30-75 bar) and Raney/Cobalt as a catalyst were carried out in water. The reaction time was monitored and increased with generations. Amino (NH_2) terminated dendrimers were isolated by evaporating the water from the filtered reaction mixture. Carboxylate terminated dendrimers were obtained by saponification of the nitrile dendrimer, by dissolving them in HCl (~40%) and refluxing for 2 h. The product was then precipitated to yield the carboxylic acid terminating dendrimer.

(DAB-dendr-$(CN)_x$ – DiAminoButane core dendrimer with x nitrile end groups)

Example 3: Synthesis of N-Chloroacetylated dendrimers (from Roy *et. al*, 1996)

Dendrimers were synthesised by solid phase peptide chemistry using 9-fluorenylmethoxycarbonyl (Fmoc) amino-protecting groups and benzotriazolyl esters as the coupling agents. The core used was L-lysine, to which the layers or generations were built. The advantage of this approach is the higher yields and well established peptide chemistry.

Dendritic L-lysine cores were elaborated with *p*-benzyloxybenzyl alcohol (Wang) resin 0.58 or 0.6 mmol/g) to which was anchored a β-alanyl spacer using the previous

Fmoc/benzotriazolyl ester strategy (Fmoc-β-Ala-OBt, 2 or 3 equiv., 0.5 equiv. DMAP, DMF, 2.5 or 3 hr). N^{α}, N^{ε}-Di-Fmoc-L-lysine were synthesised in approx. 70% yield using well established procedure with 9-fluorenylmethyl chloroformate in 10% sodium bicarbonate. The corresponding benzotriazolyl ester derivative was freshly prepared in N,N-dimethylformamide (DMF) with one equivalent each of N-hydroxybenzotriazole (HOBt) and diisopropylcarbodiimide (DIC, 0°C, then 25°C for 1 hr). In each cycle, the Fmoc-protecting groups were removed by β-elimination process using 20-25% piperidine in DMF. The degree of coupling was established spectrophotometrically by quantitation of the released dibenzofulvene chromophore at 300 nm following the piperidine treatment.

The products resulting from each sequential generation were then directly treated with pre-formed chloroacetylglycylglycine benzotriazolyl ester prepared by the above procedure. The chloroacetylglycylglycine is commercially available and did not require individual couplings of glycine residues and capping with chloroacetic anhydride as is commonly done. The completion of full derivatisation was determined by the ninhydrin test.

The ninhydrin test is used for the detection of amine groups (e.g. primary) and firstly involves the preparation of ninhydrin (using buffer, DMSO, hydridantin and ninhydrin; available as a commercial reagent), incubation at 70°C with the amine groups to be detected and quantification by colorimetric changes spectrophotometrically (570 nm). A standard calibration curve is also constructed using an amino acid such as phenyl-l-alanine. The assay is sensitive to the nano-molar range.

Using the solid phase approach, di-, tetra-, octa-, and hexadeca-valent chloroacetylated dendrimers were obtained in the first, second, third and fourth generations respectively. Structural and purity determinations were assessed by releasing the corresponding unbound chloroacetylated acid derivatives from the polymer support by treatment with aqueous trifluoroacetic acid (95% TFA, 1.5 hr). Dendrimers with yields of >90% were obtained with purity between 90-95%.

While still attached to the resin, each dendrimer generation was treated with an excess of 2-thiosialic acid derivative (1% triethylamine/DMF, 16 hr, 25°C). The dendrimers were analysed using ^{1}H-NMR and ^{13}C-NMR spectroscopy.

Examples of other branch synthetic methodologies that can be used for synthesis of dendrimers by the divergent route (but not limited to):

1 → 2 *N*-Branched

1 → 2 *N*-Branched and Connectivity

1 → 2 *N*-Branched, *Amide* Connectivity

1 → 2 *Aryl*-Branched, *Amide* Connectivity

1 → 2 *Aryl*-Branched, *Ester* Connectivity

1 → 2 *C*-Branched

1 → 2 *C*-Branched, *Amide* Connectivity

1 → 2 *C*-Branched and Connectivity

1 → 2 *C & Aryl*-Branched and Connectivity

1 → 2 *Aryl*-Branched, *N*-Connectivity

1 → 2 *Ethano*-Branched, *Ether* Connectivity

1 → 2 *Si*-Branched and Connectivity (or any other metal)

1 → 2 *P*-Branched and Connectivity

1 → 3 *C*-Branched

1 → 3 *C*-Branched, *Amide* Connectivity

1 → 3 *C*-Branched, *Amide* ('Tris') Connectivity

1 → 3 (1 → 2) *C*-Branched, *Amide* Connectivity

1 → 3 *C*-Branched, *Amide* ('Bis*homo*tris') Connectivity

1 → 3 *C*-Branched, *Amide* ('Behera's Amine') Connectivity

1 → 3 *C*-Branched and Connectivity

1 → 3 *C*-Branched, *Ether* Connectivity

1 → 3 *C*-Branched, *Ether & Amide* Connectivity

1 → 3 *N*-Branched and Connectivity

1 → 3 *P*-Branched and Connectivity

1 → 3 *Si*-Branched and Connectivity (or any other metal)

1 → 3 *Adamantane*-Branched, *Ester* Connectivity

ii) Convergent procedure

Example 4: Synthesis of polyether dendrimers (Frechet *et. al*, 1990)

An example of the synthesis of the dendrimer by the convergent approach is described in the preparation of a family of dendritic polyether macromolecules based on 3,5-dihydroxybenzyl alcohol **1** as the monomer unit. This monomer is obtained in very high yields from the formation of benzyl ethers from phenols and benzylic halides. In the example the various generation dendritic molecules will be designated by use of the following notation [G-x]-f, in which [G-x] refers to the generation number (x=0, 1, 2,...) and f refers to the functional group located at the focal point. After coupling to the core, the notation $[G\text{-}x]_n$-[C] will be used where n represents the number of dendritic fragments (generation x) coupled to the core. Starting from the benzylic bromide **2**, which is the first generation benzylic bromide [G-1]-Br, the reaction can be examined in a variety of solvents (DMF, 1,4-dioxane, THF, acetone, 3-methylbutan-2-one) and a variety of bases (Cs_2CO_3, KOH, K_2CO_3) in the presence or absence of phase-transfer agents. The optimum conditions in terms of yield and synthetic ease have been found to include the use of potassium carbonate and 18-crown-6 in refluxing acetone under vigorous stirring for 48 h. It is essential to maintain efficient stirring throughout the reaction in order to maintain a high rate of conversion. Reaction of **2** and **1** give second-generation benzylic alcohol [G-2]-OH, which can be isolated in ~90% yield after recrystallisation. The C-alkylation has been observed as a crude reaction product by high-field ^1H- and ^{13}C-NMR spectroscopy. Similarly, no C-alkylation is detected in latter generations. The reaction of [G-2]-OH with **1** gives the next-generation alcohol [G-3]OH **3** in ~88% yield after purification by flash chromatography. In this case, as with subsequent generations, it has been found that reaction with PBr_3 leads to lower yields when compared to brominations with CBr_4/PPh_3. Having obtained the third-generation bromide [G-3]-Br by reaction with **3** with CBr_4/PPh_3, it is possible to proceed to generation 4. Subsequent reactions for generation 4 lead to the higher generations up to generations 5 and 6. After high purification of the dendritic wedges, their coupling to a polyfunctional core can be carried out. The polyfunctional core is then chosen and in this example could be 1,1,1-tris (4'-hydroxyphenyl) ethane ($[C]\text{-}(OH)_3$). The individual dendritic wedges may then be fixed to a multifunctional core to create the dendrimer.

Example 5: Convergent synthesis of carbohydrate dendrimers (from Stoddart *et. al*, 1997)

Tris(hydroxymethyl)methylamine (TRIS) was used as the starting material, onto which three carbohydrate units were attached. Glucose was used as a source of the glycosyl donors towards the hydroxymethyl groups in TRIS and therefore as the carbohydrate residue present as the outer generation of the dendrimers. The free amino group in TRIS, after glycosylation, enables further elaboration through the formation of amide bonds with either branch-point synthons or, where steric problems exist, with spacer synthons possessing appropriate carboxyl functionalities. Amine functionalities are required for the branch-point and spacer synthons. Glycine (amino acetic acid) and 3,3'-iminodipropionic acid were chosen as sources of spacers and interior branch residues. Upon completion of the synthesis of the saccharide-containing dendrons, the final step was attachment of the dendrons to a multi-podent core. A 1,3,5-benzenetricarbonyl-derived unit was selected in order to provide the final dendrimer with a triply branched core.

Examples of other branch synthetic methodologies that can be used for synthesis of dendrimers by the convergent route (but not limited to):

1 → 2 *C*-Branched

1 → 2 *C*-Branched and Connectivity

1 → 2 *C*-Branched, *Ether* Connectivity

1 → 2 *C*-Branched, *Ether* Connectivity

1 → 2 *Ethano*-Branched, *Ether* Connectivity

1 → 2 *Aryl*-Branched

1 → 2 *Aryl*-Branched and Connectivity

1 → 2 *Aryl*-Branched, *Ether* Connectivity

1 → 2 *Aryl*-Branched, *Amide* Connectivity

1 → 2 *Aryl*-Branched, *Ether* and *Amide* Connectivity

1 → 2 *Aryl*-Branched, *Ether* and *Urethane* Connectivity

1 → 2 *Aryl*-Branched, *Ester* Connectivity

1 → 2 *Aryl*-Branched, *Ether* and *Ester* Connectivity

1 → 2 *Aryl*-Branched, *Ether* and *Ketone* Connectivity

1 → 2 *Aryl*-Branched, *Ethyne* Connectivity

1 → 2 *N*-Branched

1 → 2 *N*-Branched, *Amide* Connectivity

1 → 2 *C*- & *N*-Branched, *Ester* Connectivity

1 → 2 *Si*-Branched, *Silyloxy* Connectivity (or any other metal)

iii) One-step (hyperbranched) procedures (but not limited to):

1 → 2 *Aryl*-Branched

1 → 2 *Aryl*-Branched and Connectivity

1 → 2 *Aryl*-Branched, *Ester* Connectivity

1 → 2 *Aryl*-Branched, *Ether* Connectivity

1 → 2 *Aryl*-Branched, *Ether* and *Ketone* Connectivity

1 → 2 *Aryl*-Branched, *Amide* Connectivity

1 → 2 *Aryl*-Branched, *Carbamate* Connectivity

1 → 2 *Aryl*-Branched, *Urethane* Connectivity

1 → 2 *Aryl*-Branched, *Ether* and *Ester* Connectivity

1 → 2 *C*-Branched

1 → 2 *C*-Branched, *Ester* Connectivity

1 → 2 *C*-Branched, *Ether* Connectivity

1 → 2 *C*-Branched, Amide Connectivity

1 → 2 *Aryl*-Branched, C-Connectivity

1 → 2 *N*-Branched and Connectivity

1 → 3 *Ge*-Branched and Connectivity

1 → 3 (2) *Si*-Branched and Connectivity (or any other metal)

iv) Chiral dendritic macromolecules, but not limited to (Divergent procedures to chiral dendrimers):

1 → 3 *C*-Branched, *Ether* and *Amide* Connectivity

1 → 2 *C*-Branched

1 → 2 *Aryl*-Branched, *Ester* and *Amide* Connectivity

1 → 2 *Aryl*-Branched, *Ether* and *Ester* Connectivity

1 → 2 *N*-Branched and Connectivity

1 → 2 *N*-Branched and Connectivity

1 → 2 *N*-Branched, *Amide*-Connectivity

iv) Chiral dendritic macromolecules, but not limited to (Convergent procedures to chiral dendrimers):

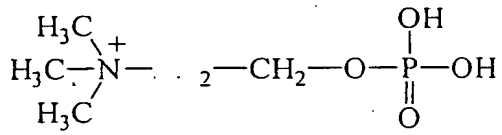
1 → 2 *Aryl*-Branched, *Ether* Connectivity

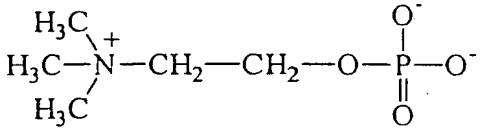
1 → 2 *C*-Branched, *Amide* Connectivity

1 → 2 *Aryl*-Branched, *Ether* Connectivity

1 → 3 *P*- and *Aryl*-Branched, *P*- and *Ether*-Connectivity

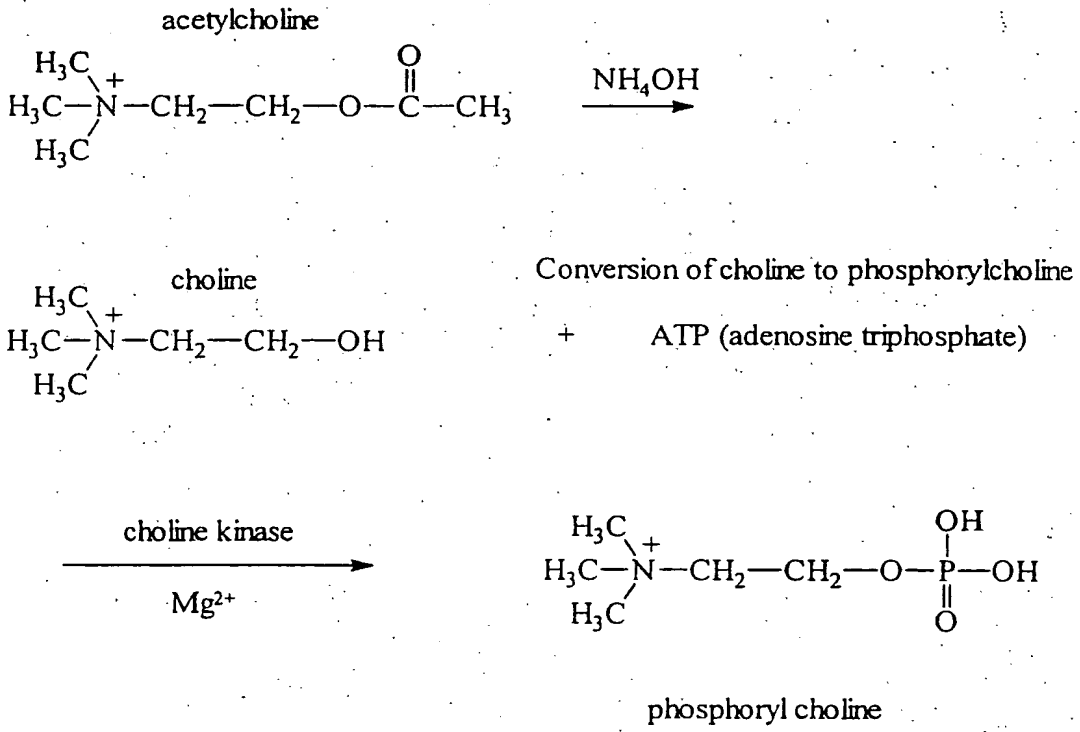
2. Phosphorylcholine





Structures of phosphorylcholine are shown above.

Phosphorylcholine can be purchased commercially (Aldrich, UK) or isolated via the following methods:



The use of acetylcholine and choline as the starting materials, reacting with choline kinase in the presence of ATP to obtain acetylcholine and phosphorylcholine as outlined in the reaction scheme above.

Choline may also be obtained by separation from tissue extracts (e.g. brain) by liquid cation-exchange chromatography using sodium tetraphenylboron. For the determination of choline, the sample is exposed directly to ATP (the ATP can also be labelled with the radioactive beta ion, ^{32}P to enable it to be detected) and choline kinase. This results in the total conversion of choline to phosphorylcholine which is then separated from the precursor by anion exchange column chromatography.

Alternatively, phosphorylcholine can also be synthesised from sphingomyelin by using sphingomyelinase, an enzyme which breaks sphingomyelin down into ceramide and phosphorylcholine.

3. Examples of Attachment or combination of support and coating

3a) Phosphorylcholine

i) Cationic polyamidoamine dendrimer generation 4 (14215 Da, 64 surface amine groups) at a concentration of 10 mg/ml was dissolved in double deionised water (DDW) and dropwise

added to a solution of phosphorylcholine (6.8 mg/ml) in DDW (ratio of 1 mole dendrimer to 64 moles of phosphorylcholine). The solution was mixed for 1 h at ambient temperature and purified by dialysis against DDW for 2 days using a dialysis membrane with a molecular weight cut off (MWCO) of 3,500 (Spectropor®) thus removing any unreacted phosphorylcholine. An electrostatic coating was thus formed between the dendrimer surface and the anionic oxygen and phosphate groups on the phosphorylcholine. A rapid and efficient purification step can also be achieved by using Amicon® ultracentrifugation using concentrators (MWCO 3,500 Da).

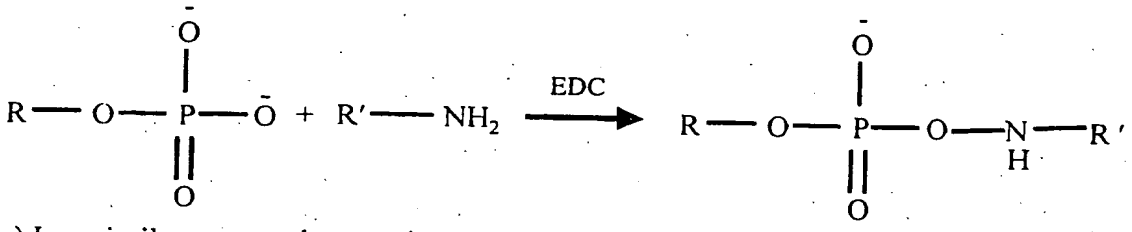
ii) A solution of the anionic polyamidoamine dendrimer generation 3.5 (12419 Da, 64 surface carboxylate groups) at a concentration of 10 mg/ml in DDW was dropwise added to a solution of phosphorylcholine (7.78 mg/ml) in DDW (ratio of 1 mole dendrimer to 64 moles of phosphorylcholine). And stirred for 1 h at ambient temperature. The resulting mixture was then purified by dialysis against DDW for 2 days using a dialysis membrane with a molecular weight cut off (MWCO) of 3,500 (Spectropor®) removing unreacted phosphorylcholine. An electrostatic coating was thus formed between the dendrimer surface and the cationic nitrogen group on the phosphorylcholine. A rapid and efficient purification step can also be achieved by using Amicon® ultracentrifugation using concentrators (MWCO 3,500 Da).

iii) Molecules containing phosphate groups may be conjugated to amine-containing molecules by using carbodiimide-mediated reaction. The carbodiimide activates the phosphate to an intermediate phosphate ester, similar to its reaction with carboxylates. In the presence of an amine, the ester reacts to form a stable phosphoramidate bond.

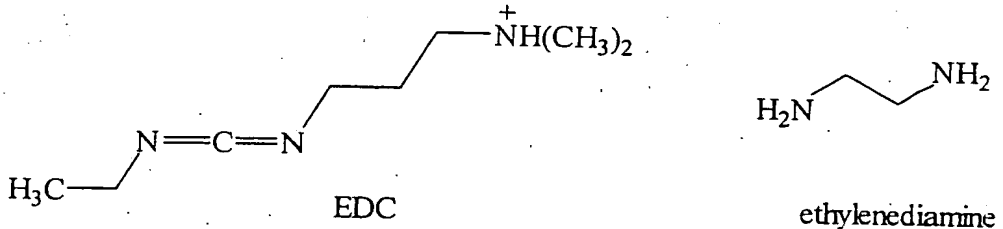
Covalent attachment of phosphorylcholine to amine terminating branched polymers was effected by dissolving phosphorylcholine in DDW to a concentration of 6.8 mg/ml. The pH was lowered to 4.5 using dilute hydrochloric acid (0.1M). An aqueous solution of EDC (8.6 mg/ml) was added and the reaction mixture was allowed to stand for 30 minutes. This would allow the EDC to activate part of the PC for linkage to the amine group on the dendrimer (i.e 1:1 reaction). The cationic polyamidoamine dendrimer generation 4 at a concentration of 10 mg/ml in DDW was then added and the pH raised or equilibrated to

between 6 and 7.5. The reaction was allowed to proceed at ambient temperature (21°C) for 3 - 4 h. Unreacted EDC is hydrolysed off as it is an unstable intermediate. The resulting mixture was purified by dialysis against DDW for 2 days using a dialysis membrane with a molecular weight cut off (MWCO) of 3,500 (Spectropor®) thus removing any unreacted phosphorylcholine, EDC or hydrolysed by-product. A rapid and efficient purification step can also be achieved by using Amicon® ultracentrifugation using concentrators (MWCO 3,500 Da). The reaction mixture can also be purified to yield the dendrimer-PC using preparative GPC using an appropriate buffer and column.

Part of the reaction is shown schematically:



iv) In a similar way to the reaction shown in iii) of the present example, the PC can be activated with EDC and coupled eventually to ethylenediamine to introduce an amine group onto the PC. After purification, a dendrimer bearing a carboxylate surface functionality can be activated with EDC once again and the PC-amine derivative introduced to couple directly onto the carboxylate terminated dendrimer, this can then be purified by dialysis or ultracentrifugation.



EDC
1-ethyl-3-(3-dimethylaminopropyl) carbodiimide

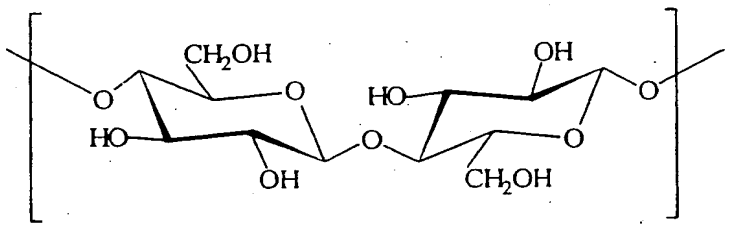
ethylenediamine

Structures of EDC and ethylenediamine

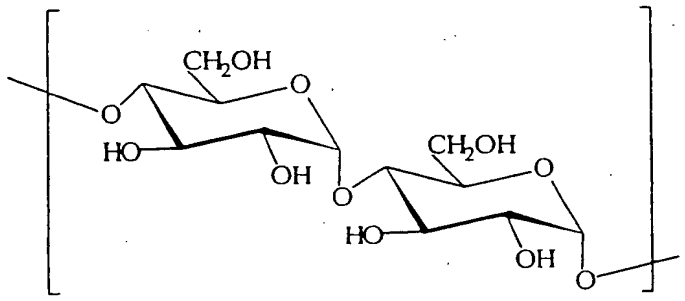
v) Synthesis of PC and coupling to dendrimer

Phosphorylcholine can be synthetically prepared according to Driver and Lewis (1999) where ethyleneglycol is added to phosphorus trichloride followed by oxidation with gaseous oxygen in boiling benzene. Once synthesised, PC can then be coupled to an amine-terminated dendrimer using EDC.

3b) Polysaccharide



Cellobiose repeating unit of cellulose; D-Glucose joined at β-1,4-linkages



Maltose repeating unit of starch (amylose); D-Glucose joined in α-1,4-linkages.

The hydroxyl and/or carboxyl residues of polysaccharides may be activated by certain substances known as activating agents, that form intermediate derivatives rendering the functional group vulnerable to nucleophilic attack. Reaction of these activated hydroxyl and/or carboxyl groups with nucleophiles such as amines results in stable covalent bonds between the carbohydrate and the amine-containing molecule (e.g. an amine terminated dendrimer). Activating agents that can be employed for this purpose include carbonyldiimidazole, certain chloroformate derivatives, tresyl- tosyl chloride, cyanogen bromide, cyanuric chloride, disuccinimidyl carbonate and various *bis*-epoxide compounds. Such activation steps are frequently done in nonaqueous solutions (i.e. dry dioxane, acetone, DMF, DMSO to prevent hydrolysis of the active species. Epoxide-containing reagents, such as the homobifunctional 1,4-(butanediol) diglycidyl ether can react with polysaccharide hydroxyl groups to form stable ether bonds.

3c) Mucopolysaccharide

Reaction of amino terminating PAMAM™ dendrimers with hyaluronan.

i) 1:1 (NH_2:COO^-) reaction to surface-coat the dendrimer:

A solution of PAMAM™ dendrimer generation 4 (0.013 g, 0.06 mmol NH_2, 14215 Da, 64 surface amine groups) in 10 ml deionised water was dropwise added to a gently stirring solution of potassium hyaluronate (0.05 g, 0.06 mmol COO^-, allowed to dissolve overnight) in 15 ml deionised water in the presence or absence of EDC and allowed to stir at ambient temperature for 1-2 h. The resulting mixture was purified with dialysis (Spectropor® membrane, molecular weight cut off of 3,500) against water for 2 days. The purified product was then concentrated by ultracentrifugation with Amicon filters (MWCO 3,500) and used to form a gel, film, a coating or cast of desired thickness.

ii) 1:<1(NH_2:COO^-) incomplete surface-coating of the dendrimer surface:

Incomplete surface-coating was achieved following the procedure above with the only exception being the number of carboxyl groups was less than 0.06 mol, depending on the amount of amino groups needed to be exposed, typically, a ratio in the range 0.5 to <1.

Thence, the free amino groups could be reacted further with another species or drug as required.

iii) 1: >1 (NH_2:COO^-) complete encapsulation with crosslinking:
Dendrimer coating and simultaneous crosslinking was achieved by following the procedure in 1 with the only exception being the number of carboxyl groups was > 0.06 mmol and typically, a ratio in the range >1 and 500. The rate of degradation in solution, 'strength' or 'hardness' of the material was relative to the rate of crosslinking. Increase in crosslinking implies decrease in the rate of degradation and increase in the strength of the product or material. In this sense, crosslinking refers to the coating of 2 or more moles of dendrimer by one mole of hyaluronan.

Reaction of carboxy-terminating PAMAM™ dendrimers with potassium hyaluronate
iv) 1:1 (dendrimer COO^- : hyaluronate OH or COO^-) reaction to surface coat the dendrimer:

A solution of PAMAM™ dendrimer generation 3.5 (0.012 g, 0.06 mmol COO^-, 12419 Da, 64 surface carboxyl groups) in 10 ml deionised water was dropwise added to a gently stirring solution of potassium hyaluronate (0.05 g, 0.06 mmol COO^-, allowed to dissolve overnight) in 15 ml deionised water in the presence of EDC (0.02 g, 0.12 mmol) and allowed to stir at ambient temperature for 1-2 h. The resulting mixture was purified with dialysis (Spectropor® membrane, molecular weight cut off of 3,500) against water for 2 days. The purified product was then concentrated by ultracentrifugation with Amicon filters (MWCO 3,500) and used to form a gel, film, a coating or cast of desired thickness.

Reaction of carboxy-terminating PAMAM™ dendrimers with chitosan
v) 1:1 (dendrimer COO^- : chitosan NH_2) reaction to surface coat the dendrimer:
A solution of PAMAM™ dendrimer generation 3.5 (0.012 g, 0.06 mmol COO^-, 12419 Da, 64 surface carboxyl groups) in 10 ml deionised water, in the presence or absence of EDC, was dropwise added to a gently stirring solution of chitosan hydrochloride (0.030 g, 0.06 mmol NH_2, 85% deacetylated) in 15 ml deionised water at pH <6 and allowed to stir at ambient temperature for 1-2 h. The resulting mixture was purified with dialysis (Spectropor® membrane, molecular weight cut off of 3,500) against water for 2 days. The purified product

was then concentrated by ultracentrifugation with Amicon filters (MWCO 3,500) and used to form a gel, film, a coating or cast of desired thickness.

vi) Gel formation

After purification, removal of the solvent/water increases the concentration and therefore increases the viscosity of the gel. The viscosity depends not only on the concentration but also on the molecular weight of the coat polymer. Naturally occurring hyaluronic acid has a molecular weight of 2,000,000 – 6,000,000 and hyaluronic acid-coated dendrimers achieve gel status and high viscosity.

vii) Film formation

After purification, the concentrated solution was poured into a petridish and allowed to dry in an oven at 50°C for 1-12 h. The thickness of the film depends on the concentration of the solution and the width of the container. Wetting with water upon allowing to reach room temperature allowed the film to come free from the glass. Alternatively the use of a teflon plate prevented the film from adhering, leading to easy isolation.

The degree of swelling in water or phosphate buffered saline (PBS, pH 7.4) ranged from 5-70% over 48 h, depending on the ratio of the reaction (dendrimer:polymer), the rate of crosslinking, thickness and solubility and degradability of the film.

General statement on generics and derivatives

The scope of the examples listed is not limited to the original or native coating, it is obvious that modifications can be made to the coating to facilitate its interaction with the support. Dextran, for example can be coated onto the dendrimer but derivatives of dextran may also be used, for example carboxymethylation of dextran to form carboxymethyl dextran or any other suitable group to facilitate specific site- or functional interactions.

4) Linking modalities

Common linkers that can be used for attachment of common end groups between the branched polymer and the coating (some modification of groups may be required to obtain the desired group before conjugation)

Modification of amines with 2-iminothiolane (Traut's reagent) to produce a sulfhydryl group.

Modification of amines with SATA (N-succinimidyl S-acetylthioacetate) to introduce a sulfhydryl group.

Modification of amines with SATP (succinimidyl acetyl-thiopropionate) as per SATA (protected sulfhydryl group).

Modification of aldehydes or ketones with AMBH (2-acetamido-4-mercaptobutyric acid hydrazide) to thiolate the aldehydes or ketones to produce sulfhydryl groups.

Modification of carboxylates or phosphates with cystamine to produce sulfhydryl groups.

Reaction of bromoethanoic acid with hydroxyl groups to yield carboxymethyl groups.

4a) Typical activating agents; Carbodiimides:

i) EDC can be used in one or two step modifications of the following groups (primarily used in aqueous reactions:

Sulfhydryls modified with ethylenimine or 2-bromoethylamine

Carbohydrates modified with diamines

Alkylphosphates with diamines

Aldehydes with ammonia or diamines

EDC is also used to activate carboxyl and hydroxyl groups

ii) N,N'-Carbonyldiimidazole (CDI)

Activation of carboxylic acids or hydroxyl groups using CDI for conjugation to other nucleophiles using zero length amide bonds or one carbon length *N*-alkyl carbamate linkages

iii) Other activating agents that can be used for coupling.

1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide (CMC)

Dicyclohexyl carbodiimide (DCC)

Diisopropyl carbodiimide (DIC)

4b) Examples of homofunctional cross-linkers

N-Hydroxysuccinimide (NHS)

Lomant's reagent [dithiobis (succinimidylpropionate)] (DSP)

Disuccinimidyl suberate (DSS)

Disuccinimidyl tartarate (DST)

Bis[2-(succinimidyloxycarbonyloxy)ethyl]sulfone (BSOCOES)

Ethylene glycol*bis*(succinimidylsuccinate) (EGS)

Disuccinimidyl glutarate (DSG)

N,N'Disuccinimidyl carbonate (DSC)

Dimethyl adipimidate (DMA)

Dimethyl pimelimidate (DMP)

Dimethyl suberimidate (DMS)

Dimethyl 3,3' –dithiobispropionimidate (DTBP)

Formaldehyde

Glutaraldehyde

Bis epoxides

Adipic acid dihydrazide

Carbohydrazide

Divinylsulphone (DVS)

1,2,3,4-diepoxybutane

dimethylolurea

dimethylolethylene urea

ethylene oxide

polyaziridine

polyisocyanate

(And other similar Linkers)

Examples of heterobifuntional cross-linkers

N-Succinimidyl 3-(2-pyridyldithio)propionate (SPDP)

Succinimidyloxycarbonyl-α-methyl-α-(2-pyridyldithio)toluene (SMPT)

Succinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (SMCC)

m-Maleimidobenzoyl-*N*-hydroxysuccinimide ester (MBS)

4-(4-N-Maleimidophenyl)butyric acid hydrazide (MPBH)

(and other similar linkers (including heterotrifunctional))

In any reaction using the water-soluble EDC, sulfo NHS can be used to stabilise the EDC-activated intermediate to allow a significantly longer coupling reaction time, as the EDC is unstable in aqueous solutions over time and elevated temperatures.

Where a pharmaceutically active agent is disclosed this can be a drug, gene, or polynucleotide.

Example of Use

Example for Gel/Film:

A viscous solution of, for example, a dendrimer-PC material according to the invention purified and is prepared in water or relevant solvent, the concentration being appropriate to the material being coated and its purpose. The viscosity of the gel depends on the molecular weight of the dendrimer. The device to be coated is dipped into the gel and then allowed to dry at room temperature forming a film/coat around the device. Further dips and consequent drying procedures will increase the amount of coat on the device. Stand-alone film is prepared by pouring the dendrimer-PC solution into a flat container, such as a petri dish, and allowing to dry in an oven. The thickness of the film depends on the concentration of the solution and the width of the container. The use of a Teflon plate prevents the film from adhering, leading to easy isolation.

Example of a bulk device:

Should the dendrimer-PC be required to synthesise the bulk of the medical device, for example, as a contact lens, the dendrimer-PC (for example) is crosslinked by reacting the terminal groups with a linker which would cause crosslinking of the dendrimer molecules. An insoluble polymeric material is formed which is of a high molecular weight. This material is then moulded or cut into shape to make a contact lens after smoothing the surface. A

generation 3.5 or generation 4 dendrimer could be used as the starting material but the process would not be limited to these generations. In an alternative embodiment the dendrimer is subjected to a cross-linking process and the PC or other biomimetic material is associated therewith subsequently.

Drug Delivery System

Example given in the case of dendrimer-PC but not limited to only dendrimer-PC.

The dendrimer-PC can be synthesied so that a pharmaceutically active agent is reversibly associated with the dendrimer or PC. This is achieved by making use of the surface functionality of the dendrimer or the unique intramoleular cavities in the core of the dendrimer. Additionally, the coating on the dendrimer, in this example PC could be used to be linked to the drug. The drug is passively linked to the dendrimer or PC through an ionic or hydrophobic interaction. Alternatively, the drug is chemically linked using a covalent bond which is degradable.

In an example the pharmaceutically active agent is a hydrophobic drug dissolved in water at a high concentration, the dendrimer solution in water is added causing the drug to enter the core of the dendrimer by diffusion. The PC is then used to coat the dendrimer with entrapped drug. A larger chemical group may also be used with the PC to provide steric hindrance to prevent immediate drug escape. The dendrimer-PC is now formed with an entrapped drug in the dendrimer core.

Slow release of hyaluronic acid

EXAMPLE

A stent or other medical tool is first coated with a denrimer by dipping the stent into a dendrimer solution. Optionally a biomimetic material such as PC is provided on the surface of the dendrimer by dipping the stent in to a solution of PC. The coated stent is then dipped into a gel containing crosslinked hyaluronic acid. The crosslinked hyaluronic acid is insoluble in water. The stent is allowed to dry and then is dipped into a solution containing

non-crosslinked hyaluronic acid which is soluble in water. This process can be repeated building up layers or crosslinked and non-crosslinked hyaluronic acid layers.

The final step should be a coating of crosslinked hyaluronic acid. When the stent is used in the body the hyaluronic acid, which has therapeutic properties will be slowly released into the body as the crosslinking degrades. The speed of delivery will depend on the strength of the crosslinking.

Bibliography

- Anderson JM and Langone JA (1999) Issues and Perspectives on the biocompatibilty and immunotoxicity evaluation of implanted controlled release systems, *Journal of Controlled Release* **57**, 107-113
- Chandy T and Sharma CP (1990) Chitosan – As a Biomaterial, *Biomat, Art. Cells, Art. Org.* **18(1)**, 1-24
- de Brabander-van der Berg EMM, and Meijer EW (1993) Poly(propylene imine) dendrimers – large scale synthesis by hetereogeneously catalysed hydrogenations, *Angewandte Chemie International English Edition* **105**, 1370-1373
- Driver M and Lewis A (1999) Preventing foul play, *Chemistry in Britain*, **35(11)**, 42-45
- Hawker C and Frechet JMJ (1990) A new convergent approach to monodisperse dendritic macromolecules, *Journal of Chemical Society Chemical Communications* **29**, 1010-1013
- Hirano S, Tanaka Y, Hasegawa M, Tobetto K and Nishioka A (1985) Effect of sulfated derivatives of chitosan on some blood coagulant factors, *Carbohydrate Research* **137**, 205-215
- Kuiper KKJ, Robinson KA, Chronos NAF, Cui JH, Palmer SJ, and Nordrehaug JE (1998) Phosphorylcholine-coated metallic stents in rabbit iliac and porcine coronary arteries, *Scandinavian Cardiovascular Journal* **32(5)**, 261-268
- Lap□ik L Jr and L Lap□ik (1998), Hyaluronan:Preparation, Structure, Properties and Applications, *Chemical Reviews* **98(8)**, 2663-2684
- Laurent TC (1998) Structure, Biology and Medical Applications of Hyaluronan and its Derivatives, Laurent TC (Ed.), Portland Press, London.
- Li Q, Dunn ET, Grandmaison EW and Goosen MFA (1992) Applications and Properties of Chitosan, *Journal of Bioactive and Compatible Polymers* **7**, 370-397
- Lim KS, Faragher RG, Reed S, Wong L, Olliff CJ, Hanlon GW, Gard P, Willis S, Denyer SP, Muir A, Lloyd AW, Khaw PT and Allan BDS (1999) Cell and protein adhesion studies in glaucoma drainage device development, *British Journal of Ophthalmology* **83(10)**, 1168-1171

- Malik N (1998) Internally supported lipid vesicle systems WO 98/56353 (PCT), December 1998 (European patent office, Munich, Germany)
- Roy R (1996) Glycodendrimers: a novel biopolymers, *Polymer News* **21**, 226-232
- Jayaraman N and Stoddart JF (1997) Synthesis of carbohydrate-containing dendrimers. 5. Preparation of dendrimers using unprotected carbohydrates, *Tetrahedron Letters* **38(38)**, 6767-6770
- Tomalia DA, Baker H, Dewald JR, Hall M, Gallos G, Martin S, Roeck J, Ryder J and Smith P (1985) A new class of polymers: Starburst-dendritic macromolecules, *Polymer Journal* **17**, 117-135
- Zheng H, Barragan P, Corcos T, Simeoni JB, Favereau X, Roquebert PO, Guerin Y and Sainsous J (1999) Clinical experience with a new biocompatible phosphorylcholine-coated coronary stent *Journal of Invasive Cardiology* **11(10)**, 608-614

CLAIMS

1. A material comprising a combination of a biomimetic material and a substance adapted to allow the biomimetic material to adopt the appropriate structural orientation to achieve maximum efficacy as a biomimetic.

2. A material as claimed in claim 1, the substance comprising a branched polymer.

3. A material as claimed in claim 1 or 2, wherein the biomimetic material is at least one of phosphorylcholine, a polysaccharide, a mucopolysaccharide or a glycosaminoglycan, syalic acid, or lectin, or a derivative or analogue of any of said biomimetic materials.

4. A material as claimed in claim 3 wherein the polysaccharide is selected from the group consisting of cellulose, starch, maltose, dextrin, dextran, an algin or alginate, or a derivative or analogue of any of said biomimetic materials.

5. A material as claimed in claim 3 wherein the mucopolysaccharide is chitin, chitosan or a derivative or analogue of any of said biomimetic materials.

6. A material as claimed in claim 3 wherein the glycosaminoglycan is hyaluronic acid, chondroitin, dermatan, keratin, or heparin, or a derivative or analogue of any of said biomimetic materials.

7. A material as claimed in claim 3, wherein the glycosaminoglycan analogue or derivative is chondroitin sulphate A, dermatan sulphate, keratin sulphate, or heparin sulphate.

8. A material as claimed in claim 3 wherein the derivative or analogue of phosphorylcholine is ceramide.

9. A material as claimed in claim 2, wherein the phosphorylcholine is an antigen or epitope.

10. A material as claimed in any one preceding claim wherein the substance is a dendrimer, an arborol, a cascade polymer, a tubular polymer, a star polymer, a hyperbranched polymer, or a hyper comb-branched polymer.

11. A material as claimed in any one of claims 2 to 10, wherein the branched polymer is cross-linked before combination with the biomimetic.

12. A material as claimed in any one of claims 1 to 10 wherein the branched polymer and biomimetic material combination is crosslinked.

13. A material as claimed in any one previous claim in the form of a hydrogel.

14. A material as claimed in any one previous claim wherein the molecular weight of the branched polymer is between 500 Da to 1 million Da.

15. A material as claimed in any one previous claim wherein a spacer/linker is introduced between the substance and the biomimetic.

16. A material as claimed in claim 15, wherein the spacer/linker is a peptide, or a polymer such as polyethylene glycol.

17. A material as claimed in any one of claims 2 to 16, wherein at least one functional group of the branched polymer is exposed.

18. A material as claimed in claim 17, wherein a polymer chain is attached to an exposed functional group on the branched polymer or an exposed group of the biomimetic material.

19. A material as claimed in any one previous claim wherein the substance and the biomimetic material are associated by covalent, van der waals, hydrophobic, electrostatic, or co-ordinate, neutral, or hydrogen bonding.

20. Use of a material as claimed in any one previous claim to coat or interact with a medical device.

21. Use of a material as claimed in any one previous claim in the delivery of a pharmaceutically active agent.

22. Use as claimed in claim 21 wherein a pharmaceutically active agent is reversibly attached to the biomimetic material.

23. Use as claimed in claim 21 wherein a pharmaceutically active agent is reversibly attached to the substance.

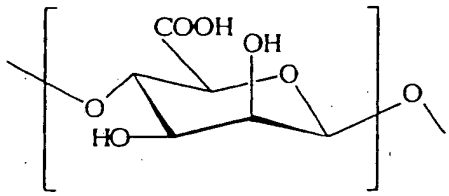
24. A medical device or tool wherein at least a portion of a surface of the device or tool is coated with the material of any one of claims 1 to 19.

25. A medical device or tool as claimed in claim 19 wherein the device or tool is selected from the group consisting of an ocular or intraocular lens, a stent, an artificial organ, or prosthetic device, pacemaker leads, artificial heart valves, vascular grafts, a limb, a glaucoma drainage device, a dialysis membrane or ultra-centrifugation membrane, a thoracic drain catheter, a vascular graft, a urological catheter or device, a guidewire, an introducer sheath, a extracorporeal circuit component, an arterial filter, heat exchanger, or a hypodermic syringe needle.

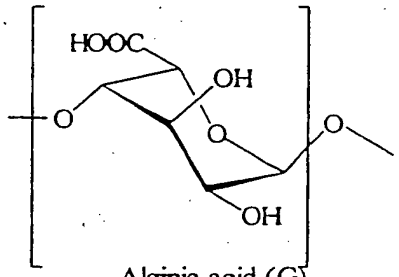
26. A method of treatment comprising the use of a medical device or tool as claimed in claims 22 or 23.

27. A method of producing a material as claimed in any one of claims 1 to 17 wherein the biomimetic material is associated with the substance in a reaction conducted in an aqueous solution or a solvent or a mixture.

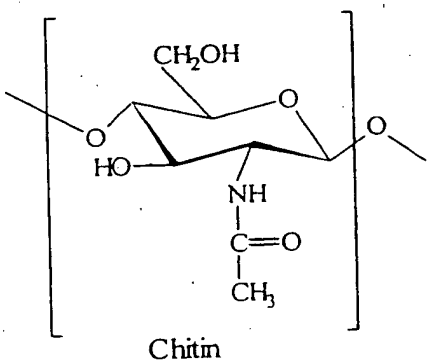
Figure 1



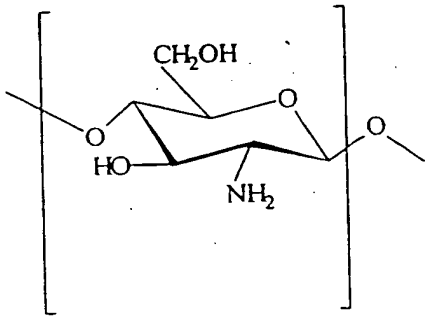
Alginic acid (M)



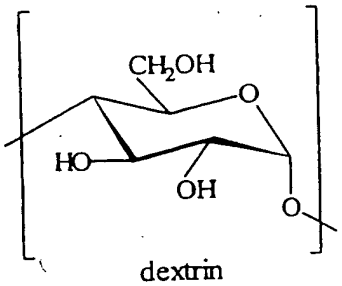
Alginic acid (G)



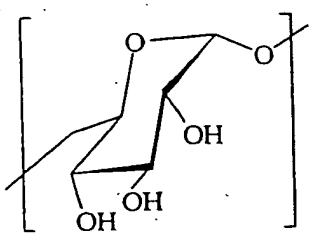
Chitin



Chitosan

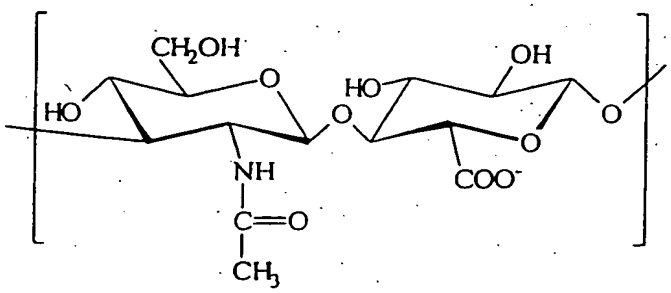


dextrin

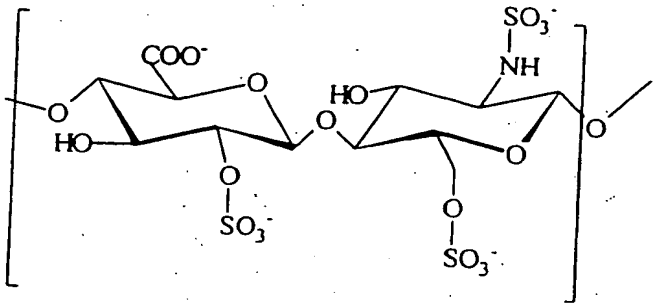


dexran

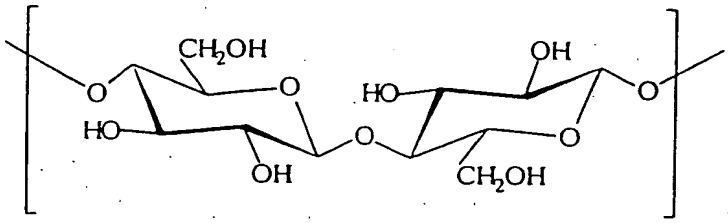
Figure 1(continued)



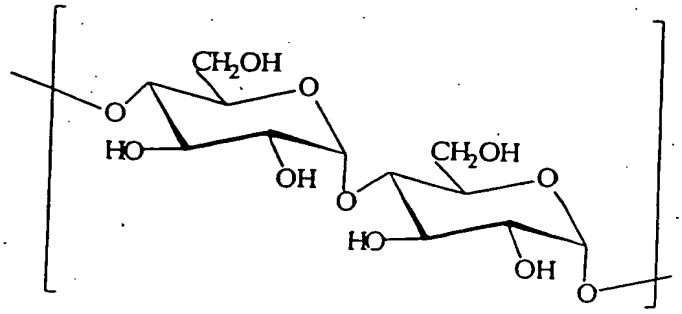
Hyaluronan



heparin



cellulose (cellubiose)



starch (maltose)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 00/04685

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61L27/34 A61L27/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ, WPI Data, BIOSIS, COMPENDEX, INSPEC, CHEM ABS Data, EMBASE, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GRIESSER HANS J ET AL: "Surface immobilization of synthetic proteins via plasma polymer interlayers" PROCEEDINGS OF THE 1998 MRS FALL MEETING - SYMPOSIUM ON PLASMA DEPOSITION AND TREATMENT OF POLYMERS;BOSTON, MA, USA NOV 30-DEC 2 1998, vol. 544, 30 November 1998 (1998-11-30), pages 9-20, XP000991364 Mater Res Soc Symp Proc;Materials Research Society Symposium - Proceedings 1999 Materials Research Society, Warrendale, PA, USA abstract page 9 -page 10 --- -/--	1-14, 17-20, 24-27

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

'A' document defining the general state of the art which is not considered to be of particular relevance

'E' earlier document but published on or after the international filing date

'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

'O' document referring to an oral disclosure, use, exhibition or other means

'P' document published prior to the international filing date but later than the priority date claimed

'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

'X' document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

'&' document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 March 2001	06/04/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Muñoz, M

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 00/04685

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 56353 A (MALIK NAVID) 17 December 1998 (1998-12-17) cited in the application the whole document	1-3, 8-13,15, 16,19, 21-23, 26,27
X	EP 0 914 835 A (ALOMONE LABS LTD) 12 May 1999 (1999-05-12) page 3, line 43 -page 4, line 4 claims	1,2,10, 11,20-27
A	ROBERTS JEANETTE C ET AL: "Preliminary biological evaluation of polyamidoamine (PAMAM) starburst-TM dendrimers." JOURNAL OF BIOMEDICAL MATERIALS RESEARCH, vol. 30, no. 1, 1996, pages 53-65, XP000992286 ISSN: 0021-9304 the whole document	
A	WO 99 10022 A (CALIFORNIA INST OF TECHN) 4 March 1999 (1999-03-04) page 12, line 4 - line 11 claims	
P,X	DE 198 49 464 A (SCHERING AG) 27 April 2000 (2000-04-27) figure 3 claims 1-6	1,2,10, 17-27

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 00/04685

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9856353 A	17-12-1998	AU 8028898 A	30-12-1998
		EP 0998267 A	10-05-2000
EP 0914835 A	12-05-1999	US 6127448 A	03-10-2000
WO 9910022 A	04-03-1999	AU 9036598 A	16-03-1999
		EP 1009451 A	21-06-2000
DE 19849464 A	27-04-2000	DE 19718339 A	12-11-1998
		AU 8015098 A	24-11-1998
		WO 9848852 A	05-11-1998
		EP 0980274 A	23-02-2000